Microscopic: Right kidney, flat epithelium, uric-acid crystals, red cells. Left kidney, round epithelium, uric-acid crystals, numerous red cells.

We have presented this rather small material because we believe that in the diagnosis of unilateral kidney lesions any contribution is of value. As we have stated above, none of the methods for determining the functional capacity of the kidneys enable us to arrive at a conclusion upon the actual function of both kidneys or either organ. In other words, none of these methods alone are sufficiently accurate to decide upon the basis of one of them the question whether a kidney should be removed or not. The real value of the methods worked out in our material lies in the comparative results and in the coincidence of the methods. A "conditio sine qua non" for the application of these methods is ureteral catheterization, and wherever feasible, catheterization of both ureters. If both urines obtained in this way are subjected to cryoscopy, phloridzin test, urea examination, and microscopic examinations, we are certainly enabled to get a fair idea upon the functional value of either kidney and to arrive at safe conclusions from our findings as to intended operative procedures.

BIBLIOGRAPHY.

1. I. Cohn. Kann der Harnleiterkatheterismus durch Harnsegregatoren ersetzt worden? Berliner klin. Wochenschr., 1903, No. 16.

2. Lichtenstern. Ueber Harnsegregatoren, Wien med. Presse, 1903, No. 13.

3. Kummell. Die neuerem Untersuchungs-methoden u. die operativen Erfolge bei Nierenkrankh., Thirty-second Congress of German Surgeons, 1903.

4. F. Bierhoff. A New Cystoscope for Simultaneous Catheterization of Ureters, etc., Medical News, March 8, 1902.

5. Michael Margulies. 200 Faelle von Katheterismus der Ureteren, Monatsber f. Urologie, 1903, No. 8.

6. S. Grim. Cryoscopy, Philadelphia Medical Journal, March 21, 1903.

7. M. Tinker. Cryoscopy, Bulletin of the Johns Hopkins Hospital, June, 1903.

8. M. Krotoszyner. Determination of the Functional Capacity of the Kidneys, etc., Occidental Medical Times, May, 1903.

9. F. S. Watson and W. T. Baily. Some Observations upon the Value of the Phloridzin Test, etc., Boston Medical and Surgical Journal, December 4, 1902.

10. Barth. Ueber funkt. Nierendiagnostik, Arch. f. klin. Chir., vol. lxxi. p. 3.

THE PRODUCTS OF GLYCOLYSIS IN BLOOD AND OTHER ANIMAL FLUIDS.

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It is a well-known fact that normal blood of almost all species of animals when freed fresh from its albumins shows a reducing body which is unquestionably largely made up of dextrose. When, however, the blood is allowed to stand some time, even when kept free from bacterial decomposition by antiseptics (thymol), this reducing body disappears, and only a substance which is able to hold copper in solution in an alkaline medium is found, but no reduction takes place. Biernocki, Pavy, and Lepine have shown further that if dextrose be added to blood under similar conditions, not only is the blood-sugar destroyed, but also a large proportion of that which has been added. To this process they give the name of glycolysis. My attention had been directed to the fact, and efforts had already been made by me to determine what became of the sugar thus destroyed, when, in 1901, P. Mayer[1] demonstrated that ox blood contained paired glycuronic acid, one of the oxidative products of dextrose, and very closely allied to it, and Mayer[2] further showed that if glycuronic acid be given to an animal and oxidation be experimentally restricted, saccharic acid is eliminated. It has also been demonstrated by the same author that if glycuronic acid or even dextrose be given in great excess, an increase of oxalic acid is found in the urine, and also that the liver is found to be surcharged with the same acid, from which he deduces the principle that if there is present an actual or relatively diminished oxidation (overwhelming the organism with carbohydrate) these partially oxidized products will appear in the urine. Based upon these facts, my efforts were directed toward determining whether the products of the so-called glycolysis of the blood might be any of these substances, viz., glycuronic, saccharic, oxalic, or carbonic acid, the final product of oxidation; of these the three last do not reduce Fehling's solution, which would account for the loss of the blood sugar in blood which is allowed to stand.

EXPERIMENT 1.—570 c.c. hydrocele fluid which had been kept two weeks on ice, was precipitated by twice its volume of 95 per cent. alcohol, filtered and washed, the alcohol driven off, acidified with acetic acid, boiled, and filtered. The resulting fluid did not show any immediate reduction with Fehling's solution, such as would be expected of dextrose. For the purpose of isolating the supposed carbohydrate body, the method of Salkowski, for obtaining xylon[3] was used, but the resulting product was a blue syrupy fluid which could not be made to crystallize, but which, when again dissolved in sodium hydrate, reduced promptly upon warming. The peculiarities of this substance suggested that it was glycuronic acid, but it was not proven. The copper suboxide formed (0.148 grams) showed 0.0667 gram of reducing body reckoned as dextrose.

EXPERIMENT 2.—500 c.c. fresh ox blood was precipitated by alcohol as before, and an effort made to isolate the reducing body by the same means as before, but ineffectually, since no precipitate could be obtained.

EXPERIMENT 3.—(*a*) 100 c.c. fresh ox blood as control for the following was precipitated with alcohol, the resulting filtrate freed from alcohol and warmed with 40 c.c. Fehling's solution. There was no immediate precipitate, but twenty-four hours afterward a whitish-yellow precipitate had formed, but it was too small in amount to furnish identification.

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[1] Zeit. f. phys. Chemie, vol. xxxi.

[2] Berlin. klin. Wochenschr., 1899, pp. 27, 28.

[3] Zeit. f. phys. Chemie, vol. xxxiv. p. 162.

(*b*) 500 c.c. of the same ox blood as above was charged with 5 grams of dextrose and allowed to remain in the same brood-oven at a temperature of 38° C. for forty-five hours, decomposition being avoided by the addition of toluol. By that time it had acquired a deep purple color, and after the toluol had been driven off over the water-bath, it was freed from albumin by alcohol, the precipitate again rubbed up with alcohol in a mortar, the alcoholic extracts united, and the alcohol removed by distillation. After establishing the volume at 1000 c.c., 50 c.c. of this clear fluid was cooked with Fehling's solution, but no reduction took place, nor was there any reaction with phloroglucin or orcin. This liquid was then allowed to stand several weeks in the refrigerator, whereupon it was filtered from the slight precipitate formed, reduced to 500 c.c. by evaporation, and then treated with subacetate of lead and ammonia; the precipitate removed, washed, suspended in water, acidified with acetic acid, and the lead removed by sulphuretted hydrogen, and the fluid reduced to 50 c.c. at 40° C. This had no reducing power, did not modify the light in the polariscope, and the remaining 40 c.c. when distilled with an equal amount of hydrochloric acid gave no furfurol with phloroglucin in the distillate; from this it is safe to say that while the dextrose was wholly converted it must have passed beyond the stage of glycuronic acid, either into a volatile substance like CO_2, or into the less readily identified carbohydrate acids.

EXPERIMENT 4.—(*a*) In order to determine whether the blood-sugar or added dextrose was converted into carbon dioxide, the final product of oxidation, as well as of yeast fermentation, 1 litre of fresh ox blood to which toluol has been added, and in a stoppered flask from which a tube led into a flask containing barium hydrate solution, which was protected from the outer air by a drying tube containing sodium hydrate, was allowed to remain in the brood-oven for twenty-four hours. When removed some insoluble barium carbonate had been formed in the adjoining flask, which was tightly stoppered and placed on ice until opportunity offered for the determination of the carbon dioxide. The blood was freed from albumin by the method of Abeles (zinc acetate and absolute alcohol), the zinc removed and the solution concentrated to 250 c.c. at 40° C. The solution had a left-turning power of 0.05 per cent. reckoned as dextrose, and gave a faint phloroglucin and orcin reaction, but no lines in the spectroscope, even when shaken out with amyl alcohol. It was now precipitated with lead and ammonia, and, after removal of the lead, concentrated to 50 c.c. Its turning power was now lost; it gave still a weak orcin and phloroglucin reaction, but no spectroscopic lines, and an osazone which was largely soluble in absolute alcohol. The barium carbonate which was formed in the barium hydrate solution was removed and the carbon dioxide determined according to Fresenius (*Quantitative Analysis*, vol. i. page 439). It amounted to 0.1054 gram in the litre of blood.

(*b*) 5 grams dextrose were added to 1 litre of the same blood as above and under the same conditions, the mixture was allowed to remain in the brood-oven the same length of time. It was then removed and treated in the same way. The resulting fluid, which was free from albumin, gave a weak phloroglucin and orcin reaction, did not reduce, but kept copper in solution in alkaline medium, and had no turning power. It was then treated as before, with lead and ammonia, and the resulting fluid concentrated to 50 c.c. It now gave a much stronger Tollens reaction, but no spectroscopic lines, and still had no turning power. The amount of carbon dioxide, determined in the same way, which had been given off was 0.1163 gram. In this case we see that 5 grams dextrose had utterly disappeared, and yet it could not have been converted into carbon dioxide, for the amount of this in the latter instance was but a trifle more than in the former, or 0.1163 gram as compared with 0.1054 gram, and in both cases it may have come from the blood itself, since Ludwig, Pfleuger, and others have found as much as 40 per cent. in dog's arterial blood.

EXPERIMENT 5.—(*a*) 5 grams dextrose were added to 250 c.c. fresh hydrocele fluid—obtained through the courtesy of Dr. Gardner W. Allen—connected with a carbon-dioxide apparatus, and the whole placed in the brood-oven for eighteen hours. It was then removed, and, without disconnecting from the apparatus, air was drawn through the blood for thirty minutes. It was then poured into an equal amount of a solution, saturated when warm with both sodium chloride and mercuric chloride, allowed to stand overnight, filtered, washed, and the filtrate and wash water freed from mercury reduced to 250 c.c. It now gave a marked phloroglucin reaction, with a line in green in the spectroscope, had a right-turning power equal to 0.5 per cent. as dextrose, and a reducing power which was equivalent to 0.66 per cent. dextrose, determined according to Allihin. Its carbon dioxide equalled 0.0804 gram.

(*b*) 10 c.c. of the same fluid taken as control and immediately precipitated, had a slight reducing power, gave no Tollens reaction, had a slight dextrorotatory power, and gave a fine, yellow, needle-like osazone, which was mostly soluble in absolute alcohol. As can readily be seen, 3.35 grams of the dextrose added to this fluid disappeared, forming no carbon dioxide, for this amount of sugar by fermentation would produce, theoretically (43.3 per cent.), 1.45 grams carbon dioxide, instead of the 0.0804 gram found, but glycuronic acid presumably, since, when paired, being a left-turner, it reduced the actual amount of carbohydrate 0.66 per cent. to 0.5 per cent. in the polariscope and gives a marked Tollens reaction.

EXPERIMENT 6.—(*a*) 1 litre fresh beef blood was placed in a flask connected with a carbon-dioxide apparatus, toluol added, and the whole placed in the brood-oven for twenty-two hours, air drawn through for thirty minutes, and then freed from albumin by sodium and mercuric chloride, and the latter removed. The resulting clear fluid was then reduced to 50 c.c., which had no turning power, but a reducing power equal to 0.0586 gram of dextrose, or 11 per cent. The remainder was then cooked in an autoclave with 1 per cent. sulphuric acid for one hour and neutralized, whereupon it acquired a dextrorotatory power of less than 0.1 per cent. It formed an osazone of lemon-colored needle-like crystals mixed with globules of a darker hue, which were almost entirely soluble in absolute alcohol. The original blood emitted 0.1878 gram carbon dioxide.

(*b*) To another litre of the same blood 5 grams dextrose were added in a flask which was connected with the carbon-dioxide apparatus, and the whole placed in the brood-oven as before for the same time. It was then freed from albumin and reduced to 50 c.c. It was levogyr to 0.1 per cent., and had a reducing power equivalent to 0.0591 gram, or 0.118 per cent. as dextrose. The remainder was now cooked with sulphuric acid, but it remained a left-turner and gave a marked phloroglucin and orcin reaction without spectroscopic lines. It gave an osazone consisting of a few crystals (needles), but chiefly of globules, which were soluble in absolute alcohol. Its carbon dioxide amounted to 0.9564 gram.

(*c*) 1 litre of the same blood was precipitated at once and reduced to 50 c.c. It then had a turning power of +0.1 per cent. and a reducing power of 0.0403 gram as dextrose, or 0.08 per cent. It formed an osazone soluble in alcohol, consisting of irregular, feathery masses.

In both (*a*) and (*b*) the fact that the rotatory power was less than the reducing power and that (*a*) was converted to a dextrogyr body and that (*b*)'s levogyr power was very much lessened by splitting with sulphuric acid shows that at least a portion of the reducing substance was in the form of glycuronic acid, a fact still further emphasized by the marked orcin and phloroglucin reaction in (*b*), which, based upon its reducing power and compared with the control (*c*), had lost all of the dextrose added. A part of this may have been converted to carbon dioxide, since there was a marked increase in this over (*a*), to which no dextrose was added. As is well known, the paired glycuronic acid being a left-turner, diminishes or obliterates the dextrogyr power of dextrose.

EXPERIMENT 7.—(*a*) 1 litre of a pleuritic exudate, obtained through the courtesy of Dr. Mackechnie, guarded against decomposition by toluol, was placed in the brood-oven for twenty hours, removed, freed from albumin by the mercury process, and reduced to 200 c.c. The rotatory power was now +0.1 per cent. and the reducing 0.23 per cent. as dextrose. There was no Tollens reaction.

The fluid was then cooked with sulphuric acid, after which it still retained a rotatory power of +0.1 per cent.; Tollens weak. In producing the osazone in this and all the following experiments a strict proportion between the phenylhydrazin added and the amount of reducing body, as determined by the polariscope after splitting, was maintained, so that one molecule of the reagent was added to one molecule of the suspected glycuronic acid, which, according to their molecular weights, gave 0.55 gram of the reagent to 1 gram of the substance sought, or as my reagent was in the form of the chloride, 0.735 gram was used. Another improvement which was suggested by Neuberg was to place the substance and reagent in a brood-oven for twelve to fourteen hours instead of boiling it, and this proved very satisfactory in increasing the product and avoiding tenacious masses, which would form on the edges of the flask in the old way. This limited addition of phenylhydrazin is supposed to cause the glycuronic acid to form an osazone to the exclusion of dextrose, or, if the latter does form, it can be separated by washing with hot water and absolute alcohol. It is true that Neuberg and Mayer use bromphenylhydrazin, but I think I have demonstrated that the ordinary reagent can be used in the same way.

(*b*) 10 grams of dextrose were added to 1 litre of the same exudate and the whole placed in the brood-oven for the same time as before, then freed from albumin and reduced to 200 c.c. Polariscope showed +0.7 per cent. and reduction 1.201 per cent., while there was no phloroglucin and no orcin reaction. After cooking with sulphuric acid, rotatory power 0.75 per cent. as dextrose, both orcin and phloroglucin positive, and osazone of well-formed globules of dark color.

(*c*) 400 c.c. same exudate freed at once from albumin and reduced to 50 c.c. Polariscope showed +0.2 per cent. and reduction 0.35 per cent. After splitting polariscope showed +0.25 per cent., but there was no Tollens reaction. Attention must be called here to these general features repeated in the other experiments; the reduction is greater than the polariscopic reading in (*a*) and (*b*); the Tollens reaction is brought out strongly by the splitting with sulphuric acid, as is also an increase in the polariscopic reading. It becomes evident, also, that during the process of evaporation, even if the reaction is kept slightly acid, as was the custom, a partial splitting of the paired acids takes place, else we should much oftener come across levogyr bodies. The separation of the glucosazone from the combination of glycuronic acid and other acids with phenylhydrazin could be accomplished with repeated washings with hot water and after drying with absolute alcohol upon the filter paper. The wash water and alcohol upon cooling would often cause a precipitation of a crystalline nature, which consisted almost entirely of glucosazone, and its needles are very different from the globules which the other compounds form. The demonstration of the nature of the precipitate, which is insoluble in hot water and absolute alcohol, was most difficult. Since the glucosazon is soluble in absolute alcohol, presumably the residue which was left was an hydrazin of glycuronic or saccharic acid, but no solvent could be found which would enable one to determine its power in the polariscope. Mayer and Neuberg use a mixture of absolute alcohol and pyridine, and its left-turning power is so great (0.2 gram in 10 c.c. of the mixture gives a reading of —7° 25′) that it can be easily distinguished from all other osazones or hydrazins, but the pyridine could not be procured, and after various attempts to find a solvent in mixtures of glacial acetic acid, acetone and absolute alcohol, the attempt to identify the substance in this way was abandoned. It suffices to say, however, that the insoluble portion had all the characteristics of the hydrazin of glycuronic acid described by Mayer.[1]

Another fact worth mentioning is that the product obtained with phenylhydrazin from the normal blood was perfectly soluble in hot water and alcohol, while that from both bloods subjected to the brood-oven temperature was only partially so. In no case was there sufficient evidence that more than an infinitesimal amount of the lost dextrose was converted to the carbohydrate acids, and we must fall back upon the suggestion made that the dextrose may combine with the albumin of the blood and be removed with that.

The next point to establish was whether the dextrose could have been converted to oxalic acid by the process of glycolysis, as is assumed by Mayer, in the animal when oxidation is impaired. For this purpose four portions of fresh ox blood were taken and treated in the following manner:

(*a*) 1 litre was precipitated at once by mercuric and sodium chlorides, the mercury removed, and, keeping the steadily increasing acid reaction in check by sodium carbonate, the whole was reduced to 200 c.c. It was then shaken three times with ether, no hydrochloric acid having been added on account of that present from the mercuric chloride removed, and an effort made to determine the oxalic acid, if present, according to the method of Salkowski; but only the merest traces of this substance were found too small for estimation. The remaining watery solution was freed from ether and reduced to 50 c.c., when its turning power was found to be +0.2 per cent., while by the method of Allihin there was found to to be 0.199 per cent., or practically the same as shown by the polariscope. It gave no reaction with phloroglucin. It was then split with 1 per cent. sulphuric acid in a closed flask, and the volume having been re-established, with due calculation for the amount used in the reduction determination, it now gave a turning power of +0.15 per cent., and afforded, dried at 110° C., 0.0888 gram of an osazone which dissolved in 3.5 c.c. glacial acetic acid, gave a turning power of —0.2

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[1] Zeit. f. Physiol. Chemie, 29, 67.

per cent. reckoned as dextrose. From all the evidence found here probably nothing but dextrose existed in the blood.

(*b*) 1 litre of blood, to which 10 grams of dextrose were added, was attached to a water-pump, after having been treated with toluol, in hopes of at least partially preventing decomposition, and air was drawn through it for twenty-four hours. It was then freed from albumin in the same way, reduced to 200 c.c., and shaken three times with ether, in order to extract the oxalic acid, but only traces were found. The ether was driven off from the watery solution, and the whole reduced to 50 c.c. Its turning power was now +1.875 per cent., and it gave the phloroglucin but not the orcin test. By the Allihin method with copper it showed 1.54 per cent. of a reducing body; a peculiarity in the reduction was noticed which is suggestive of glycuronic acid, as well as other substances apart from dextrose. The Fehling solution became at first greenish when boiled, and a yellowish precipitate was thrown down, in which were seen particles of the red suboxide of copper. Owing to the fact that on evaporation a large amount of salts had been thrown out of solution and the possibility that some dextrose was mechanically held by this mass from which it had been filtered, these were extracted with water until the last water showed no reduction. The volume had now risen to 225 c.c., giving a polariscopic reading of +0.51 per cent., which was cooked in a closed stone flask for eight hours with 1 per cent. sulphuric acid. This solution, when neutralized, possessed a turning power of +0.425 per cent. and both the phloroglucin and orcin reactions, but no lines. It also gave, dried at 110° C., 1.244 grams of an osazone or hydrazin, consisting of microscopic rosettes and globules, which was insoluble in hot water and absolute alcohol, whose optical properties could not be determined, as before stated, for want of a suitable solvent. In this, as in previous instances, an attempt was made to determine the nitrogen by Kjeldahl, but, though the destruction of the substance by the acid seemed complete, no ammonia could be distilled over; yet it is not safe to judge from this that no nitrogen existed in the compound.

(*c*) This litre of blood, treated with toluol, was allowed to remain at brood-oven temperature for twenty-four hours without the addition of dextrose, freed from albumin, reduced to 200 c.c., an almost neutral reaction having been maintained, shaken out with ether, freed from the same, concentrated to 50 c.c., and tested for reducing bodies. No oxalic acid was found beyond the mere traces mentioned in the other samples. It showed no reduction, no turning power and phloroglucin test, hence no further attempts were made to isolate an osazone. It simply proves what has been so often established, that a blood loses its native dextrose, even when by strong antiseptics the possibility of fermentation is practically excluded, without leaving any traces of discoverable oxidative or glycolytic products of the dextrose which has disappeared.

(*d*) 1 litre of blood, to which was added 10 grams of dextrose and sufficient toluol to cover surface of the fluid, was placed in a brood-oven for twenty-four hours and treated as before. No oxalic acid was found, but it gave a marked phloroglucin reaction. The polariscope showed, in 100 c.c. of fluid, 0.503 per cent., while by reduction of copper and weighing, 0.5 per cent. was found. The remainder after these tests was diluted again to 100 c.c., showing a turning power of +0.15 per cent., and after splitting with 1 per cent. sulphuric acid, +0.2 per cent. It also gave marked phloroglucin and orcin reactions, as also 0.370 gram of an hydrazin, insoluble in hot water and absolute alcohol. Here we have no decided increase of reduction over polariscopic reading, as would be present were the levogyr paired glycuronic acid present to any extent, nor do we find a marked increase of turning power after splitting with acid, as would be expected under the same conditions. We do, however, find an insoluble hydrazin and Tollens reaction, particularly after splitting, in both cases where dextrose has been added. Taken all in all, there is evidence that paired glycuronic acid exists in ox blood, as demonstrated by Mayer, and human serum, but none to substantiate the conversion of dextrose into that substance by means of any glycolytic ferment existing in the blood. We think that we have also demonstrated that the large amount of dextrose failing to reappear in these experiments is not converted into carbonic acid or oxalic acid. Where this change takes place, if it does, as insisted upon by Mayer, we do not know. One thing is evident to everyone who has worked with blood: that our means of separating the albumin are crude, and that the enormous coagulum or precipitate formed by the albumin must of necessity carry with it a considerable portion of the blood-sugar which cannot be separated from it. The problem of what becomes of the sugar in glycolysis still remains to be solved.

The methods of isolating the rarer forms of the oxidative products of dextrose, like glycuronic and saccharic acids, are still so crude that they offer but little hope of success in this direction. A glycolytic enzyme undoubtedly exists in the liver, but its action has been demonstrated on glycogen only, and can probably have nothing to do with the oxidation of dextrose. Croftan also claimed to have found an enzyme in the leukocytes which would destroy dextrose, but, beyond demonstrating that the sugar disappeared, nothing further was proved. Salkowski suggests that the oxidase which is found in the tissues may be the active agent, but, as far as I can learn, this agent has only been able to oxidize the more highly oxidizable bodies, like benzaldehyde and salicyldehyde, but never dextrose. The proof of the former action has been established by the detection of benzoic and salicylic acids; but, as far as can be learned, never of the latter by the detection of glycuronic, glycuric, saccharic acids, etc., the oxidative products of dextrose.